REMARKS

Upon entry of the Amendment, claims 1-10 are pending in the application. Claims 1-3, 6, 8, and 9 have been amended. Claim 10 is new. Support for new claim 10 is found in the specification, such as on page 30. Therefore, no new matter has been added.

I. Objections to the Specification

The Examiner has objected to the Abstract and to the use of a hyperlink in paragraph [0137] at page 66, lines 14-21.

Applicants respectfully submit that the amendments to the Abstract and the specification overcome these objections. The Abstract is in narrative form. Further, the specification presently does not use a hyperlink.

II. Claim Objections

Claims 1 and 3-9 are objected to because of the following informalities:

> Claims 8 and 9 are objected to under 37 C.F.R. § 1.75(c) as being in improper form because they reference back to another multiple dependent Claim 6;
>
> Claims 1 (claims 3-5 dependent therefrom) and 6 (7 dependent therefrom) are objected to because the recitation of "Atk2" should be in parenthesis and follow the phrase it abbreviates when used for the first time; and
>
> Claim 3 is objected to because the recitation of "coding" should be improved with respect to clarity; the Examiner suggests the phrase -- encoding --.

With respect to claims 8 and 9, these claims presently do not reference back to another multiple dependent claim. Claim 6 presently is not multiply dependent.

With respect to claim 1, the Examiner asserts that the claim 1 should recite the full name of "Atk2" followed by its abbreviation in parenthesis. Claim 1 presently recites "Akt-homolog-2 ("Akt2")." The name "Akt2" is based on the meaning viral-akt-homolog-2. v-akt is an oncogene deduced from AKT8 which is a retrovirus isolated from a spontaneous thymoma in the AKR mouse. Applicants provide herewith as support Proc. Natl. Acad. Sci. U.S.A., 1987 Jul; 84(14): 5034-5037.

With respect to claim 3, the claim recites the phrase --encoding--, as suggested by the Examiner.

III. Claim Rejections - 35 U.S.C. § 101

Claims 1-3 have been rejected under 35 U.S.C. § 101 allegedly because the claimed invention is directed to non-statutory subject matter.

The Examiner asserts that the inventions recited in claim 1-3 do not distinguish themselves from the natural form thereof.

Claims 1-3 have been amended to further clarify that the polypeptide or polynucleotide has been "isolated." In this regard, the inventions recited in claims 1-3 distinguish themselves from the natural form thereof.

IV. Claim Rejections - 35 U.S.C. § 112

(A) Claims 1-7 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite.

The Examiner asserts that the phrase amino acid sequence "represented by" is unclear. Claims 1, 2, 6 and 8 have been amended so that the phrase "the amino acid sequence represented by" reads --the amino acid sequence of--.

Similar to the claim objection, the Examiner asserts that the phrase "Akt2" in claim 1 is unclear. For the same reasons provided above, Applicants respectfully submit that amended claim 1 is now definite.

With respect to claim 6, the Examiner asserts that the phrase "binds to Akt2, with Akt2" is unclear. Claim 6 has been amended to delete the phrase "with Akt2."

(B) Claims 1-7 have been rejected under 35 U.S.C. § 112, first paragraph, written description, as allegedly failing to comply with the written description requirement.

Claim 1 presently recites an amino acid sequence in which from 1 to 10 amino acids are deleted, substituted and/or inserted in the amino acid sequence represented by SEQ ID NO:2 or SEQ ID NO:4, and which binds to Akt2.

Claim 6 presently recites an amino acid sequence having a homology of 90% or more with the amino acid sequence represented by SEQ ID NO: 2 or SEQ ID NO: 4 and which binds to Akt2.

The Examiner asserts that the amino acid sequences of SEQ ID NOs: 2 and 4 are insufficient to provide written description support for (i) any amino acid sequence in which 1 to 10 amino acids are deleted, substituted and/or inserted and binds to any Akt2 or (ii) any amino acid sequence having 90 % homology and binds to any Akt2. The Examiner notes that there is no disclosure regarding the structure of the homologues, nor which amino acids might be

modified such that homologs would maintain its ability to bind to any Akt2. The Examiner concludes that the claims lack written description due to the absence of disclosure of a correlation between function and structure of SEQ ID NOs: 2 and 4, or disclosure of any homologs.

Applicants respectfully traverse.

The specification provides sufficient guidance and support that a person skilled in the art can reasonably predict which homologs would provide for the binding to Akt2 and have a homology of 90 % or more. Example 1 describes that the mouse AKBP2 encoding the amino acid sequence of SEQ ID NO: 2 binds to human Akt2. Example 6 describes that the human AKBP2 encoding the amino acid sequence of SEQ ID NO: 4 binds to human Akt2. A person skilled in the art can compare SEQ ID NO: 2 and SEQ ID NO: 4 to determine the regions that provide for binding to Akt2. Page 30 of the specification describes the parameters and the programs that can provide for the comparison. A person skilled in the art can also look to the parameters and programs described at page 30 of the specification to determine the homology of the homolog.

Moreover, with respect to claim 1, the specification provides sufficient guidance and support to predict an amino acid sequence in which from 1 to 10 amino acids are deleted, substituted and/or inserted in the amino acid sequence represented by SEQ ID NO:2 or SEQ ID NO:4, and which binds to Akt2. An amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4 that has 10 amino acids thereof modified has about 98.3 % homology with, respectively, SEQ ID NO: 2 or SEQ ID NO: 4. As described above, page 30 of the specification provides for the

parameters and programs for comparing the homolog to SEQ ID NO: 2 or SEQ ID NO: 4. In this regard, a person skilled in the art can predict the homologs having about 98.3 % homology with either SEQ ID NO: 2 or SEQ ID NO: 4 and that bind to Akt2.

(C) Claims 1-7 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement.

The Examiner asserts that while the specification is enabling for a peptide consisting of SEQ ID NOs: 2 and 4, it does not reasonably provide enablement for the homologs recited in claims 1 and 6. Referring to page 12 of the Office Action, the Examiner asserts that the specification does not establish:

> (A) regions of the protein structure which may be modified without affecting Akt2 binding of a polypeptide consisting of SEQ ID NO: 2 or 4;
>
> (B) the general tolerance of a polypeptide consisting of SEQ ID NO: 2 or 4 to modification and extent of such tolerance;
>
> (C) a rational and predictable scheme for modifying any amino acid residue of a poluypeptide consisting of SEQ ID NO: 2 and 4 with an expectation of obtaining the desired binding; and
>
> (D) sufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

For the same reasons provided above, Applicants respectfully submit that the specification provides sufficient guidance and support so that a person skilled in the art can make and use the amino acid sequences of the homologs recited in claims 1 and 2.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the

Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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Molecular Cloning of the akt Oncogene and Its Human Homologues AKT1 and AKT2: Amplification of AKT1 in a Primary Human Gastric Adenocarcinoma

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Medical Sciences

Molecular cloning of the *akt* oncogene and its human homologues *AKT1* and *AKT2*: Amplification of *AKT1* in a primary human gastric adenocarcinoma

(AKT8 retrovirus/protooncogene/gene amplification)

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ABSTRACT A previous report described the isolation of a directly transforming retrovirus, AKT8, from a spontaneous thymoma of an AKR mouse. The AKT8 provirus has now been molecularly cloned from a transformed, nonproducer cell line. The virus genome contains both viral and nonviral, cell-related sequences; the nonviral sequence has been designated v-*akt*, the presumed viral oncogene of the AKT8 virus. This gene lacks homology to the 16 other oncogenes tested. The cloned provirus has undergone a partial deletion, during cell passage *in vitro*, that prevents direct demonstration of the transforming ability of this molecular clone. Two human homologues of the v-*akt* oncogene, *AKT1* and *AKT2*, were cloned. A survey of 225 human tumors for changes involving *AKT1* led to the discovery of a 20-fold amplification of this gene in one of the five gastric adenocarcinomas tested. The results demonstrate that AKT8 has the characteristic structure of a directly transforming retrovirus and that it contains a gene derived from highly conserved cellular sequences that may be involved in the pathogenesis of some human malignancies.

A molecular dissection of those genetic and cellular events that result in tumor formation has become possible with the identification of cellular genes that can contribute to the malignant phenotype. This advance in our ability to understand and analyze tumorigenesis resulted from discoveries in several research areas; particularly, the identification, cloning, and further analysis of cellular genes that have been acquired by directly transforming retroviruses (1), and that are responsible for their ability to cause tumors, have provided researchers with a set of potentially oncogenic genes that can be tested for alterations in naturally developing tumors. Oncogenes have been shown to be altered by amplification, chromosomal translocation, insertions, and base changes in a variety of experimental and spontaneously developing tumors in both humans and animals. This report describes the identification of a retrovirus-associated oncogene, v-*akt*, and shows that its cellular homologue is altered in a spontaneous human tumor.

The AKT8 murine retrovirus was isolated 10 years ago during virological studies on tumor cell lines established from spontaneous thymomas of the high-leukemia AKR mouse strain (2). The virus was identified by its ability to produce foci of malignant transformation in the mink lung epithelial cell line CCL 64. A transformed cell line free of replication-competent helper virus was isolated, showing that the transforming virus is defective, as are other directly transforming murine retroviruses. The viral genome was shown to produce a polyprotein that contained both viral and nonviral antigenic specificities (3). In the case of other transforming retroviruses, such nonviral antigenic specificities are due to the acquisition of cellular sequences by the virus. These altered cellular sequences are responsible for the transforming ability of the virus. Thus, it seemed possible that the AKT8 virus had also acquired oncogenic sequences of cellular origin in exchange for viral genetic material, accounting for its replication incompetence, polyprotein production, and oncogenic ability.

The *in vitro* biology of the AKT8 virus is unique. The only cell line found to be sensitive to focus formation upon infection is the CCL 64 mink lung epithelial cell line. That a variety of other cell lines, including the NIH 3T3 fibroblast line, are negative for transformation by the AKT8 virus suggested that the virus might contain a previously undescribed oncogene. The oncogene, v-*akt*, has now been molecularly cloned and analyzed. Hybridization analysis indicates that v-*akt* is unrelated to the other oncogenes tested. Cloning of the human homologues of the *akt* gene, human *AKT1* and *AKT2*, permitted identification of an amplification of an *AKT1* allele in a primary gastric adenocarcinoma. These results strongly support the involvement of *akt* in the development of some human malignancies.

MATERIALS AND METHODS

Cell Lines. The mink (CCL 64, obtained from the American Type Culture Collection) and mink(AKT8) cell lines were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. AKT8-transformed mink cells were cloned in the same medium to which low-melting-point agarose had been added to 0.3%. For transformation tests, mink cells were seeded at 2×10^5 cells in 60-mm Petri dishes and infected after 24 hr with AKT8 virus in the presence of Polybrene (1 μg/ml). The medium was changed the next day and every 2–3 days thereafter for the next 14 days, at which time the plates were scored for the presence or absence of foci.

Virus. AKR ecotropic retrovirus (AKV) purified by banding in a sucrose gradient was obtained from Electro-Nucleonics (Silver Spring, MD) and was a gift from the late Wallace P. Rowe (National Institutes of Health, Bethesda, MD). Viral RNA was prepared by detergent lysis of virions in the presence of proteinase K followed by sucrose gradient centrifugation and selection on an oligo(dT)-cellulose column.

DNA. DNA was extracted from cell lines and fresh tumors by suspending the cells or finely minced tissue in 50 mM Tris Cl, pH 8.0/200 mM NaCl/10 mM EDTA with 0.5% NaDodSO$_4$ and 0.1 mg of proteinase K per ml. After overnight digestion at 37°C, the DNA was extracted once with phenol and twice with chloroform, precipitated with 2 volumes of ethanol, and resuspended in 10 mM Tris Cl, pH 7.4/1 mM EDTA. For restriction enzyme analysis, DNA (10 μg) was

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Abbreviation: AKV, retrovirus from AKR mice.

digested overnight with the indicated enzyme under conditions recommended by the supplier, and the resulting fragments were separated by agarose gel electrophoresis. Conditions for blotting and hybridization analysis with ^{32}P-labeled probe were as described by Southern (4). Radioactive probes were prepared by nick-translation of DNA (5) or by cDNA synthesis with reverse transcriptase using random primers on purified viral RNA (6).

Molecular Cloning. Procedures for molecular cloning were similar to those described in a standard handbook (7). The Charon 21A vector (8) was used to clone the AKT8 genome. The human homologues *AKT1* and *AKT2* were selected from a human genomic library prepared with the Charon 28A vector (9) and obtained from P. Leder (Harvard University, Cambridge, MA). All phage stocks were plated on, or grown in, *Escherichia coli* LE392.

RESULTS

The strategy for cloning the AKT8 viral genome relied on the detection of murine retroviral sequences in the nonproducer cell line mink(AKT8). A ^{32}P-labeled AKV cDNA prepared from gradient-purified virus was used as a probe to examine sequences in mouse and mink cells (Fig. 1). A cross-hybridizing band at 7.0–7.5 kbp is present in mink(AKT8) cell DNA but is absent from DNA of the parental mink cells. AKR mouse DNA shows the expected multiple copies of endogenous virus and virus-related genomes that have been described previously (10).

A library was initially prepared using the Charon 21A vector and *Eco*RI-digested mink(AKT8) DNA selected for sizes between 6 and 9 kbp. Screening of this library, and of several subsequent libraries similarly prepared, failed to identify the desired sequences. The cloning was complicated by the presence of mink endogenous retroviral sequences that cross-hybridized with the probe and that had to be isolated and analyzed before being discarded. It is possible that the AKT8 genome could not be cloned from mink(AKT8) cells because of "poison" sequences that prevented growth of the desired recombinant phage in the bacterial host. Therefore, genomic DNA was digested with *Eco*RI, size-selected, partially digested with *Mbo* I, and ligated into Charon 21A that had been restricted with both *Eco*RI and *Bam*HI. This procedure was designed to randomly eliminate sequences that might be inhibiting growth of the recombinant. This strategy was apparently successful, since a clone containing murine retroviral sequences (as determined by much stronger hybridization to mouse DNA than to mink DNA when this clone was used as a probe) was isolated.

The structure of the AKT8 clone is shown in Fig. 2. Hybridization analysis of this clone demonstrated sequences that cross-hybridized with AKV flanking nonreactive sequences of 1000–1500 bp, which we named v-*akt*. The 5'→3' orientation of the clone was determined by hybridization with subfragments of cloned AKV proviral DNA (11). Of note is the *Pst* I–*Kpn* I–*Sma* I configuration characteristic of murine ecotropic retroviral long terminal repeats present at the 3' end of the viral-probe-reactive sequences (12). This same configuration of enzyme sites should also be present at the 5' end of the virus clone but is missing. The reason for this discrepancy was explained by further biological experiments.

Attempts to use the cloned AKT8 proviral DNA to transform mink cells and thus prove the oncogenicity of these sequences failed. These negative results prompted a reevaluation of the cell line from which the AKT8 genome had been cloned. It had become increasingly difficult to recover high titers of transforming virus from the mink(AKT8) cell line. Recloning of these cells in agar yielded a new line, mink-(AKT8)cl1, which produced an order-of-magnitude greater yield of transforming virus after superinfection with helper murine retroviruses. Also, the recloned line had a 10-fold higher cloning efficiency in agar than the original mink(AKT8) clone. These results suggested that there was loss of part or all of the AKT8 provirus from a portion of the mink(AKT8) cells. Southern blot analysis of mink(AKT8) and mink(AKT8)cl1 cell DNA was performed to investigate this question. When the 0.8-kbp 5' *Pst* I–*Pst* I fragment was used as a probe, two fragments, of 0.8 and 2.0 kbp, were identified in the mink(AKT8) cells but only a single, 2-kbp fragment was detected in the mink(AKT8)cl1 cells. Thus, the AKT8 genome that was cloned had undergone a partial deletion, probably between a point within the 5' long terminal repeat and v-*akt*, during *in vitro* passage of the mink(AKT8)

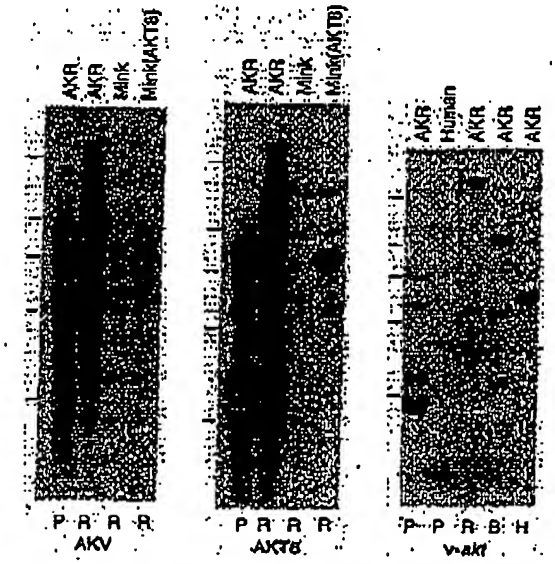

FIG. 1. Southern blot analysis of AKT8 proviral and *akt* proto-oncogene sequences in AKR mouse, mink, and mink(AKT8) cell DNA and human placenta DNA. Probes were AKV cDNA, the 3.1-kilobase-pair (kbp) *Pst* I fragment from the AKT8 provirus clone (Fig. 2), and the *akt*-specific subclone pvakt/1 (*Left*, *Center*, and *Right*, respectively). Tick marks indicate the positions of size markers (*Hin*dIII fragments of bacteriophage λ DNA) in the following order (from top to bottom) 23.1, 9.4, 6.6, 4.4, and 2.3 kbp. Genomic DNA was digested with restriction enzymes as indicated below each lane: P, *Pst* I; R, *Eco*RI; B, *Bam*HI; H, *Hin*dIII.

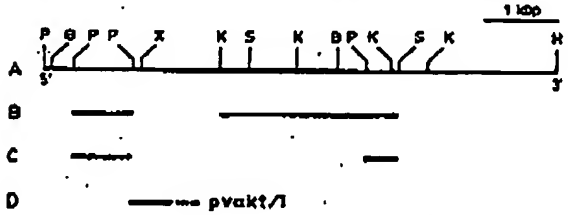

FIG. 2. (*A*) Restriction enzyme map of the molecularly cloned AKT8 retrovirus. (*B*) Fragments that hybridized with the AKV cDNA probe. (*C*) Fragments that hybridized with a probe against the viral long terminal repeat. (*D*) Diagram of a probe containing only nonviral sequences. Dotted line indicates that portion of the *Pst* I–*Kpn* I fragment which was digested with BAL-31 exonuclease until no further crossreactivity with viral sequences was detectable. Restriction enzyme sites are as follows: P, *Pst* I; B, *Bam*HI; X, *Xho* I; K, *Kpn* I; S, *Sma* I.

cells. Reversion to a nontransformed phenotype may have been favored by an increased adherence of reverted cells to plastic flasks in comparison with AKT8-transformed cells. The identification of this defective genome also explained the inability to demonstrate transformation with the cloned DNA.

To further examine the nonviral sequences, a v-*akt* probe (pvakt/1) that derived from this region of the AKT8 genome and that did not hybridize to AKV DNA was cloned. As shown in Fig. 1, an AKT8 probe containing both v-*akt* and viral sequences hybridized to the AKT8 genome present in mink(AKT8) cells and also to three other fragments that were also present in the parental cells. This finding would be consistent with the presence of a highly conserved, cell-derived sequence in the AKT8 genome. The pvakt/1 probe hybridized to only several fragments in AKR mouse DNA. The most conclusive evidence for the nonviral nature of the v-*akt* sequences, however, was obtained after the isolation of a homologous mouse genomic DNA clone (isolated from a BALB/c genomic library carried in a λ phage vector) that showed no crossreactivity with retroviral sequences when tested with a complete AKV ecotropic probe. The extent of conservation of *akt* sequences throughout evolution was shown by the crossreactivity of the murine v-*akt* probe with human DNA (Fig. 1). This degree of evolutionary conservation has not been reported for any murine retrovirus but is characteristic for oncogenes. v-*akt* was tested against the following oncogene DNAs and failed to hybridize to any of them: *abl*, *erb*, *fes*, *fms*, *fos*, *mos*, *myc*, N-*myc*, *myb*, *raf*, Ha-*ras*, Ki-*ras*, N-*ras*, *rel*, *sis*, and *src*. (N-*myc* and N-*ras* DNAs were of human cellular origin; the remainder were of viral origin. Oncogene DNAs were obtained as recombinant plasmids from the American Type Culture Collection.)

To isolate the human *akt* gene(s), a human genomic library was screened with the pvakt/1 probe. Clones from two apparently distinct loci, *AKT1* and *AKT2*, were isolated. *AKT1* contained sequences that reacted with probes from both the 5′ and the 3′ ends of v-*akt*, whereas *AKT2* reacted only with a 3′ v-*akt* probe. Restriction maps of these clones are shown in Fig. 3. The 5′→3′ orientation of *AKT1* is by analogy with v-*akt*. A probe for *AKT1* was isolated by subcloning the 5′ 4.1-kbp *Bam*HI–*Bam*HI fragment (phakt-1/1). phakt-1/1 identified only the *AKT1* gene on Southern blots of human DNA.

To evaluate the possible role of *AKT1* in human malignancy, DNA that had been extracted from fresh human tumors was surveyed by Southern blotting. DNA digested with *Bam*HI gave one of three patterns due to the presence of a *Bam*HI allelic polymorphism detected with the phakt-1/1 probe. A 4.1- and an 11.1-kbp fragment occurred with approximately equal frequency in the population surveyed, and tumor material either was homozygous for one or the other allele or was heterozygous, containing both fragments. Fig. 4 is a screening blot that demonstrates an amplification of *AKT1* in a poorly differentiated adenocarcinoma of the

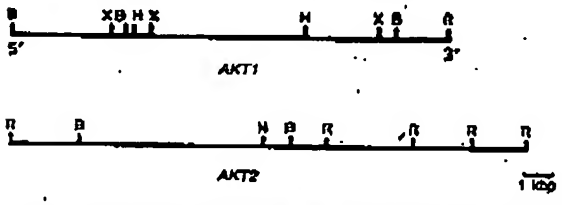

FIG. 3. Restriction enzyme maps of the human *akt* loci *AKT1* and *AKT2*. The orientation of *AKT1* was determined by comparison with the known orientation of AKT8 viral DNA. Restriction enzyme sites are as follows: B, *Bam*HI; R, *Eco*RI; X, *Xho* I; H, *Hin*dIII.

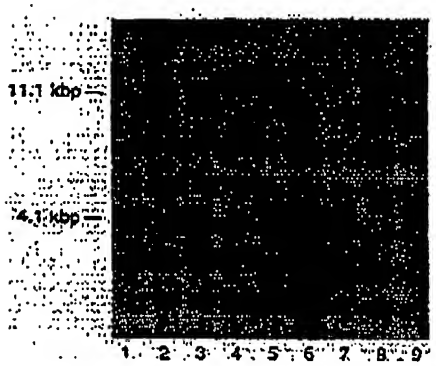

FIG. 4. Analysis of tumor DNAs for gene dosage of the *AKT1* locus. DNA extracted from fresh tumor specimens obtained at surgery was digested with *Bam*HI and analyzed by Southern blot hybridization with phakt-1/1 as probe. Lanes: 1, liposarcoma; 2, lung large-cell carcinoma; 3, colon adenocarcinoma; 4, breast adenocarcinoma; 5, lung adenocarcinoma; 6, gastric adenocarcinoma with amplified 4.1-kbp *Bam*HI allele of *AKT1*; 7, parathyroid adenoma; 8, endometrium carcinoma; 9, lung squamous-cell carcinoma.

stomach (lane 6). The 11.1-kbp *Bam*HI allele serves as an internal standard for the amplified 4.1-kbp allele in this heterozygous patient. The patient was newly diagnosed and untreated and had locally metastatic tumor at the time of gastrectomy. He died several months later with widespread metastatic disease. DNA obtained from his uninvolved stomach tissue was found to contain only a single copy of each *AKT1* allele. The titration shown in Fig. 5 indicates an ≈20-fold amplification of *AKT1* in the tumor. The β-globin gene (*HBB*) is not amplified, whereas the c-*myc* gene (*MYC*) is amplified 5-fold in the tumor.

DISCUSSION

The identification and molecular cloning of the *akt* oncogene, and the demonstration of its alteration in a human tumor, parallel the analysis of other oncogenes carried by retroviruses. The results make it clear that the directly transforming AKT8 retrovirus has acquired cellular sequences. A transformed, nonproducer cell line makes a fusion polyprotein with both viral and nonviral antigenic specificities from the AKT8 virus genome. The cell-derived AKT8 sequences that are encoded in the viral polyprotein are almost certainly responsible for the malignant transformation of the indicator mink cells. AKT8-infected mink cells are capable of anchorage-independent growth and grow subcutaneously in nude mice (S.S., unpublished results). The inability to demonstrate transformation of mink cells by DNA transfection is explained by a partial deletion in the 5′ portion of the AKT8 genome. Although this final proof of the oncogenicity of the cloned AKT8 genome is lacking—pending cloning of intact viral sequences—the biology of the virus *in vitro*, its genome structure, and its amplification in a spontaneous human tumor serve to establish the oncogenic potential of the *akt* gene.

The biology of the AKT8 virus is unique, suggesting that the *akt* oncogene may have a novel mode of action. Unlike many other directly transforming retroviruses, including those carrying *ras* and kinase-related oncogenes, AKT8 does not transform murine fibroblasts or any other cell line tested. The mink cell line CCL 64 is a rare, nontransformed epithelial cell line. Identification of the *akt* protein product and its function in the cell should allow the determination of the cause of the apparent cell-type specificity of AKT8 transfor-

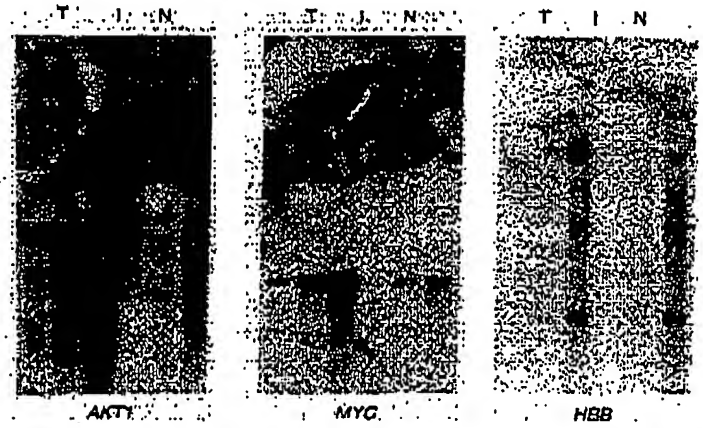

FIG. 5. Gene-dosage analysis of the gastric adenocarcinoma with amplification of *AKT1*. DNA from the tumor (T) and adjacent normal (N) stomach was digested with *Bam*HI for analysis of the *AKT1* gene, *Sst* I for analysis of the human c-*myc* gene (*MYC*), and *Bam*HI for analysis of the β-globin gene (*HBB*). Three lanes, with 0.6, 2.5, and 10 μg, were loaded for each DNA. Probes were phakt-1/1 for detection of *AKT1* sequences, a previously described 5′ subclone of *MYC* (pMC41 3RC, obtained from R. Dalla-Favera; ref. 13) for detection of *myc* sequences, and a β-globin cDNA (obtained from H. Kazazian, Johns Hopkins University, Baltimore; ref. 14) for detection of *HBB*.

mation *in vitro*. The lack of hybridization to other oncogenes, as well as preliminary chromosome mapping in mice (S.S. and C. Kozak, unpublished data) and humans (S.S., K. Huebner, C. M. Croce, N. Z. Parsa, and J. R. Testa, unpublished data), indicates that *akt* is a previously unreported oncogene.

The AKT8 virus was isolated from a cell line established from a spontaneous thymoma occurring in the AKR mouse strain, a strain selected for a high incidence of thymic leukemia/lymphoma (15). Thymomas arise as a result of a persistent viremia developing shortly before birth and due to vertically transmitted endogenous retroviral genomes that are activated to produce infectious virus (16). Viral insertion events have been shown to occur near a known oncogene, *myc*, or in locations that are in the vicinity of presumed oncogenes in some AKR thymomas (17–19). In preliminary work to examine the role of *akt* in the pathogenesis of AKR thymomas, the pvakt/1 probe detected a 3.5-kilobase transcript in blot-transfers of electrophoretically fractionated total RNA from thymus and spleen. Examination of RNA extracted from five thymomas showed a transcript size and quantity of *akt* no different from that of normal thymus. This result and a preliminary analysis of thymoma DNAs indicate that alterations involving *akt* are not a consistent feature of AKR thymomas. Recent studies on the induction of tumors in mice with AKT8 virus (S.S. and J. Hartley, unpublished data), however, suggest that *akt* is involved in the pathogenesis of some AKR thymomas.

The finding of an amplified *AKT1* allele indicates that this gene may be involved in the pathogenesis of some human malignancies. High-grade gene amplifications are rare events except in certain tumors such as neuroblastoma (20) and small-cell lung cancer (21). The survey of human tumors in the present work revealed only one instance of such amplification among 225 diverse human malignancies consisting of both solid and hematopoietic tumors. The lack of available fresh tissue prevented examination of *AKT1* gene expression or chromosome analysis, in this case. Five other gastric adenocarcinomas examined contained only single copies of *AKT1*. Thus, amplification of *AKT1* is a sporadic event in gastric adenocarcinoma—as would be expected from the relative rarity of reports of high-grade oncogene amplification in the common human malignancies.

I am indebted to the late Dr. Wallace P. Rowe for his support and encouragement in the early phase of these studies. I am also grateful to Dr. Joseph Eggleston, Dr. Risa Mann, and the residents of the Johns Hopkins Pathology Department for their help in obtaining tumor specimens. This research was supported by National Institutes of Health Grant CA26089, a Leukemia Society special fellowship, a grant from the State of Maryland American Cancer Society, and an American Cancer Society institutional grant.

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